



## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification <sup>6</sup> :  A61K 39/395, C07K 16/00		A1	(11) International Publication Number: <b>WO 98/23289</b>  (43) International Publication Date: 4 June 1998 (04.06.98)
(21) International Application Number: PCT/US97/21437  (22) International Filing Date: 26 November 1997 (26.11.97)		(81) Designated States: CA, JP, European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).	
(30) Priority Data: 60/031,607 27 November 1996 (27.11.96) US		<b>Published</b> <i>With international search report.            Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>	
(71) Applicants: THE GENERAL HOSPITAL CORPORATION [US/US]; 55 Fruit Street, Boston, MA 02114 (US). BRANDEIS UNIVERSITY [-/US]; P.O. Box 9110, Waltham, MA 02254-9110 (US).			
(72) Inventors: ISRAEL, Esther, Jacobowitz; 19 Alden Street, Newton, MA 02159 (US). SIMISTER, Neil, E.; 415 South Street, Waltham, MA 02254 (US).			
(74) Agent: FRASER, Janis, K.; Fish & Richardson, P.C., 225 Franklin Street, Boston, MA 02110-2804 (US).			

(54) Title: MODULATION OF IgG BINDING TO FcRn

## (57) Abstract

Disclosed are mutant IgG molecules having altered amino acid sequences in the FcRn-binding region. These changes confere increased or decreased affinity for FcRn and thus, respectively, a decreased or increased rate of clearance from the systemic circulation. Such molecules can be attached to detectable labels or cytotoxic moieties for imaging tissues or for delivering cytotoxins. Also disclosed is a method for identifying IgG molecules with altered half-lives in circulation by contacting the molecules with FcRn.

***FOR THE PURPOSES OF INFORMATION ONLY***

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	France	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Latvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav Republic of Macedonia	TM	Turkmenistan
BF	Burkina Faso	GR	Greece	ML	Mali	TR	Turkey
BG	Bulgaria	HU	Hungary	MN	Mongolia	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MR	Mauritania	UA	Ukraine
BR	Brazil	IL	Israel	MW	Malawi	UG	Uganda
BY	Belarus	IS	Iceland	MX	Mexico	US	United States of America
CA	Canada	IT	Italy	NE	Niger	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NL	Netherlands	VN	Viet Nam
CG	Congo	KE	Kenya	NO	Norway	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NZ	New Zealand	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's Republic of Korea	PL	Poland		
CM	Cameroon	KR	Republic of Korea	PT	Portugal		
CN	China	KZ	Kazakhstan	RO	Romania		
CU	Cuba	LC	Saint Lucia	RU	Russian Federation		
CZ	Czech Republic	LI	Liechtenstein	SD	Sudan		
DE	Germany	LK	Sri Lanka	SE	Sweden		
DK	Denmark	LR	Liberia	SG	Singapore		
EE	Estonia						

- 1 -

MODULATION OF IgG BINDING TO FcRn

Background of the Invention

The field of the invention is immunoglobulins.

5        Immunoglobulin G (IgG) is used intravenously to treat a number of diseases that involve immune deficiencies, including acquired immune deficiency syndrome (AIDS), idiopathic thrombocytopenic purpura (ITP), Kawasaki disease, Guillaine-Barre Syndrome, and 10 dermatomyositis. Recently, there has also been increasing use in immunosuppression in transplanted patients, and in specifically directed antibody therapy, such as monoclonal antibodies used as a form of cancer chemotherapy.

15      Previously, immunoglobulins were extracted from pooled whole blood, but the increasing risk of infection from AIDS and the related decrease in the available blood supply precipitated increased reliance on murine monoclonal antibodies for *in vivo* human therapy.

20      Initially, antibodies from such sources had problems with short half-life and inciting an immune response against the murine proteins (the HAMA response), but both of these problems have been alleviated somewhat by the practice of humanizing the antibodies by combining the 25 human constant region with the mouse variable region.

Although monoclonal antibodies now have a half-life similar to that of native human IgG, there are still situations where it would be desirable to have even more control over the length of time before immunoglobulins 30 are catabolized.

This is especially true in treating immune deficiency conditions. When the concentration of IgG is increased above normal levels in the circulation, its half-life decreases. Therefore it is difficult to 35 maintain higher-than-normal levels of the immunoglobulins during treatment, and patients require frequent

- 2 -

injections of the antibodies. On the other hand, in situations where the antibodies are being used as a means to target a chemotherapeutic agent, such as a radionuclide or a protein toxin, to a particular tissue 5 or cell type, the dosage is often limited by the risk of damage to the bone marrow and other normal tissues due to non-specific binding. In such cases, a shorter-than-normal half-life would be desirable.

Summary of the Invention

10 The invention is based on the discovery that FcRn, in binding to IgG, sequesters it and protects it from degradation. FcRn is a receptor found on the intestinal surface of the neonate, and is responsible for the shuttling of maternal milk IgG from the intestinal lumen 15 through the intestinal epithelial cell into the systemic circulation. It is now known also to be responsible for preventing IgG from being cleared from the animal's circulation. Using the guidance provided herein, one can create, using recombinant methods, an IgG molecule that 20 has one or more amino acid additions, deletions, or substitutions (conservative or nonconservative) in the region that binds to FcRn, thereby either increasing or decreasing the molecule's affinity for FcRn. An increase in affinity would translate into the altered IgG's having 25 a longer half-life *in vivo* than native IgG, while a decrease in affinity for FcRn would have the opposite effect.

Although monoclonal antibodies now have a half-life similar to that of native human IgG, there are still 30 situations where it would be desirable to have even more control over the length of time before immunoglobulins are catabolized.

This is especially true in treating immune deficiency conditions. When the concentration of IgG is

- 3 -

increased above normal levels in the circulation, its half-life decreases. Therefore it is difficult to maintain higher-than-normal levels of the immunoglobulins during treatment, and patients require frequent 5 injections of the antibodies. On the other hand, in situations where the antibodies are being used as a means to target a chemotherapeutic agent, such as a radionuclide or a protein toxin, to a particular tissue or cell type, the dosage is often limited by the risk of 10 damage to the bone marrow and other normal tissues due to non-specific binding. In such cases, a shorter-than-normal half-life would be desirable.

An IgG with an increased half-life *in vivo* would be useful in treating conditions such as acquired immune 15 deficiency syndrome (AIDS) or idiopathic thrombocytopenic purpura (ITP), where maintaining a higher-than-normal concentration of circulating IgG is desirable. A mutant IgG molecule that binds less strongly to FcRn and is therefore cleared more rapidly would be of benefit where 20 the IgG is being used for chemotherapy or as a tumor imaging agent.

The mutated IgG of the invention would have amino acid substitutions in the FcRn-binding region only, resulting in altered half-life only, with no substantial 25 change in overall immune function.

Also claimed is a method of removing IgG from the blood of an animal by administration of soluble FcRn, which would complex with the circulating antibody and prevent it from being sequestered by cellular FcRn. This 30 method would be especially useful in chemotherapies, to control the ratios of tumor-bound to circulating antibodies.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as 35 commonly understood by one of ordinary skill in the art

- 4 -

to which this invention belongs. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, the preferred methods and materials are 5 described below. All publications, patent applications, patents and other references mentioned herein are incorporated by reference in their entirety. In case of conflict, the present specification, including definitions, will control. In addition, the materials, 10 methods, and examples are illustrative only and not intended to be limiting.

Other features and advantages of the invention will be apparent from the following detailed description, and from the claims.

15

Brief Description of the Drawings

Figure 1 is a graph illustrating clearance of intravenously injected  $^{125}\text{I}$ -immunoglobulins in mice with and without  $\beta 2$ -microglobulin. Approximately  $1 \times 10^7$  counts per minute of labeled IgG was injected into the 20 external jugular vein. Blood was collected at the time points indicated. Percent initial radioactivity = counts per minute per milligram blood at indicated time point  $\times$  100 per counts per minute per milligram blood at  $t_0 = 1$  minute after injection. Mouse IgG1 is represented by the 25 solid line, chicken IgY by a dashed line,  $\beta 2\text{m}^{+/+}$  by circles,  $\beta 2\text{m}^{+/-}$  by triangles, and  $\beta 2\text{m}^{-/-}$  by diamonds;  $n = 5$  for each group.

Figure 2 is a diagram illustrating partial amino acid sequences of IgG molecules used in this study (EU 30 numbering). Alternative amino acid residues within an isotype are shown below the most common sequence. Sequences are from Kabat et al., 1991, Sequences of proteins of immunological interest, 5th edn., pg. 683,

- 5 -

NIH, Bethesda, MD. Predicted contacts with FcRn (from Burmeister et al., 1994, *Nature* 372:379) are underlined. Residues for binding to FcRn (Kim et al., 1994, *Eur. J. Immunol.* 24:2429; Raghavan et al., 1995, *Biochemistry* 5 34:14649) are marked with an asterisk.

Figure 3 is an illustration depicting the proposed role for FcRn in protecting IgG from degradation. IgG taken up in the fluid phase binds FcRn at acidic pH in early endosomes. IgG bound to FcRn is recycled to the 10 plasma membrane and released, whereas unbound proteins are sorted to lysosomes and degraded.

#### Detailed Description

The altered IgG's of the invention are most readily prepared by standard recombinant DNA methods, 15 e.g. site-directed mutagenesis or PCR. The regions of IgG to be mutated corresponds to amino acids 248 through 257, 308 through 314, and 429 through 436 of IgG. Within these regions, five particular amino acid residues have been identified as being important in FcRn binding, but 20 others can be explored as well by using DNA primer-based site-directed mutagenesis, available commercially as a kit (Amersham, Arlington Heights, Illinois), and well known in the art. The Amersham kit can be used according to the manufacturer's instructions in order to mutate 25 specific residues within the FcRn-binding region.

Preliminary *in vitro* comparison of the binding of non-native IgG molecules relative to native IgG is done by radiolabelling the IgG molecules, incubating them with cells which express FcRn, washing the cells, and then 30 measuring the amount of radioactivity that remains in association with the cells. Suitable cells include endothelial cells, cells (such as human embryonic kidney cell line 293) transfected with a vector encoding FcRn,

- 6 -

or intestinal epithelial cells from suckling rats or mice. Alternatively, one could use a brush border fraction derived from such intestinal epithelial cells, prepared in accordance with standard methods (e.g.,  
5 Wallace and Rees, 1980, Biochem. J. 188:9).

The mutant IgG antibodies can be tested for *in vivo* half life by radiolabelling with Na<sup>125</sup>I (DuPont, Wilmington, DE) using the Iodogen™ method (Pierce Biochemical, Rockford, IL). Free iodine is removed by  
10 gel filtration on Sephadex G-25 and aggregated immunoglobulins are removed by gel filtration on Sephadex G-200. The <sup>125</sup>I-immunoglobulin can then be diluted in 10% normal mouse serum to an injection concentration of 1 x 10<sup>7</sup> cpm / 150 µl. Anesthetized mice can be injected in  
15 the jugular vein with 150 µl of the radiolabelled immunoglobulin, and subsequently bled with capillary tubes from the retro-orbital sinus at serial time points following injection. Plasma would be collected by centrifugation and total radioactivity measured in a  
20 gamma counter and expressed as cpm/mg blood. The percentage radioactivity remaining in the blood after the last bleed would be calculated relative to the value 1 minute after injection. Protein-bound radioactivity would be measured by precipitation of the plasma in 10%  
25 TCA. The clearance curves for the various radiolabelled antibodies can then be plotted, revealing which produce antibodies with the desired characteristics. To test whether immune function is preserved, immunoprecipitation or ELISA assays can be used to ensure that the antibody  
30 does in fact complex with the antigen of interest.

For administration to human patients, the mutated immunoglobulins of the invention can be humanized by methods known in the art, e.g., monoclonal antibodies can be commercially humanized (Scotgen, Scotland; Oxford  
35 Molecular, Palo Alto, CA). The antibodies can then be

- 7 -

purified using known methods, such as absorption onto immobilized Protein A or immunoaffinity chromatography. Following purification, the antibodies of the invention can be administered to patients in a pharmaceutically acceptable excipient such as physiological saline. The antibodies of the present invention can be administered by any standard route including intravenously, intraperitoneally, intramuscularly, or subcutaneously. It is expected that the preferred route of administration will be intravenous.

As is well known in the medical arts, dosages for any one patient depends on many factors, including the patients general health, sex, size, body surface area, age, as well as the particular compound to be administered, time and route of administration, and other drugs being administered concurrently. Determination of correct dosage for a given application is well within the abilities of one of ordinary skill in the art of pharmacology.

The invention also includes screening methods for identifying IgG molecules with altered circulating half-lives and binding affinities relative to native IgG.

Example 1: Increased clearing in mice that lack  $\beta$ 2-microglobulin.

Animals. Mice heterozygous for the  $\beta$ 2m gene disruption (Zijlstra et al., 1990, Nature 344:742) were mated and allowed to deliver. Tail tissue was taken from the pups for genomic DNA preparation to determine their  $\beta$ 2m genotypes (Israel. et al., 1995, J. Immunol. 154:6246). The clearance experiments were done at 8 weeks of age. The mice were given 0.01% NaI in their drinking water one day prior to injection and throughout the period of monitoring clearance of the  $^{125}\text{I}$ -immunoglobulins.

- 8 -

**Preparation of  $^{125}\text{I}$ -immunoglobulin for injection:** Mouse IgG1, IgG2a, IgG2b and IgG3 (Cappel, Durham, N.C.) and chicken IgY (Cappel) were labeled with  $\text{Na}^{125}\text{I}$  (Dupont, Wilmington, DE) using the Iodogen™ method (Pierce Biochemical, Rockford, IL). Free iodine was removed by gel filtration on Sephadex G-25 and aggregated immunoglobulins were removed by gel filtration on Sephadex G-200. Precipitation in 10% trichloroacetic acid (TCA) showed that at least 90% of the  $^{125}\text{I}$  in preparations of IgG1, IgG3, and IgY was bound to protein, and at least 80% of the radiolabel in IgG2a and IgG2b was bound to protein. The  $^{125}\text{I}$ -immunoglobulin was diluted in 10% normal mouse serum to achieve approximately  $1 \times 10^7$  cpm/150  $\mu\text{l}$  for injection.

**Clearance experiments:** Under pentobarbital anesthesia (65  $\mu\text{g/g}$  body weight), the external jugular veins of  $\beta 2\text{m}^-/-$ ,  $\beta 2\text{m}+/-$ , and  $\beta 2\text{m}+/+$  mice were exposed and injected with approximately 150  $\mu\text{l}$  of the  $^{125}\text{I}$ -immunoglobulin diluted in 10% normal mouse serum. The mice were bled with capillary tubes from the retro-orbital sinus at serial time points following injection, under light isotharine anesthesia. Plasma was collected by centrifugation and total radioactivity was measured in a gamma counter and expressed as cpm/mg blood. After the last bleed, the animals were killed with  $\text{CO}_2$ . The percentage radioactivity remaining in blood was calculated relative to the value 1 min. after injection. Protein-bound radioactivity was measured by precipitation of the plasma in 10% TCA. This protocol has been approved by the Institutional Animal Care and Use Committee at Brandeis University.

**Pharmacokinetic data analysis:** The data from each animal were fitted to a double exponential model,

$$C = e^{b-k_2 t} + e^{a-(k_1+k_2)t},$$

- 9 -

by a non-linear least squares method with a multiplicative error structure. The parameters  $k_1$  and  $k_2$  were constrained to be positive. The area under the curve at infinity ( $AUC_\infty$ ), mean residence time (MRT), 5 terminal elimination half life ( $t_{1/2}$ ), and the phase I half life were then calculated from a, b,  $k_1$ , and  $k_2$ . Means and s.e.m.s for these parameters were calculated for each genotype group. Differences between the groups were tested with an analysis of variance using Gabriel's 10 procedure to make pairwise comparisons (Gabriel, 1978, J. Amer. Statistical Assoc. 73:724).

**Results:** The clearance curves for radiolabeled mouse IgG1 and chicken IgY are shown in Figure 1, which is a graph showing the clearance of intravenously injected 15  $^{125}\text{I}$ -labelled immunoglobulins in mice with and without  $\beta 2$ -microglobulin. The curves are biphasic, with phase 1 representing equilibration between the intravascular and extravascular compartments and phase 2 representing the elimination of the protein from the intravascular space. 20 The pharmacokinetic parameters are shown in Table 1. The phase 1 half lives did not differ significantly for IgG1 and IgY, or between the three  $\beta 2\text{m}$  genotypes. However, there were significant differences in the phase 2 (terminal) half lives ( $t_{1/2s}$ ). Mouse IgG1 was degraded 25 more rapidly in  $\beta 2\text{m}^{-/-}$  mice than in  $\beta 2\text{m}^{+/-}$  or  $\beta 2\text{m}^{+/+}$  littermates. Specifically, the half life was 25 hours in the  $\beta 2\text{m}^{-/-}$  animals compared to 5 days for the  $\beta 2\text{m}^{+/+}$  mice (Fig. 1). There was no significant difference 30 between the clearance in heterozygous ( $t_{1/2} = 123$  h) and wild type mice, suggesting that the effect on IgG catabolism was not related to the dose of the  $\beta 2\text{m}$  gene. The radioactivity left in the serum was 95-98% precipitable by TCA at all time points (data not shown), suggesting the radioactivity measured was indeed bound to 35 IgG. There was little release of TCA-soluble

- 10 -

radioactivity into the systemic circulation during the time period studied.

Radiolabeled chicken IgY was cleared equally rapidly in wild type mice and in mice homozygous for the disruption in the  $\beta 2m$  gene (Figure 1). The terminal half life of IgY (21-22 hours) was not significantly different than that of mouse IgG1 I in  $\beta 2m-/-$  mice (Table 1).

Table 1. Pharmacokinetic parameters of  $^{125}I$ -IgG clearance.

$\beta 2m$ genotype (n)	Ig isotype	$AUC_{\infty}^a$ $\times 10^7$	MRT <sup>b</sup> (hours)	Phase I half life (hours)	$t_{1/2}^c$ (hours)
+ / + (2)	mIgG1 <sup>d</sup>	35 (8) <sup>f</sup>	167 (6)	3.1 (0.1)	120 (3)
+ / - (4)	mIgG1	33 (3)	168 (14)	6.7 (1.7)	123 (10)
- / - (4)	mIgG1	8.3 (0.9)	29.7 (3.6)	5.5 (0.4)	25 (3.6)
+ / + (3)	cIgY <sup>e</sup>	3.7 (0.2)	15.7 (0.8)	5.4 (0.6)	22 (1.2)
- / - (3)	cIgY	3.8 (0.1)	18.7 (3.0)	4.6 (1.2)	21 (0.5)

a = Area under the curve

b = Mean residence time

c = Terminal half life

d = Mouse IgG1

e = Chicken IgY

f = Mean (s.e.m.)

Pharmacokinetic parameters for the clearance of mouse IgG1, IgG2a, IgG2b and IgG3 compared in a separate experiment in  $\beta 2m-/-$ , +/- and +/+ mice are shown in Table 2. The half lives of IgG1, IgG2a and IgG3 were significantly lower in  $\beta 2m-/-$  mice than in  $\beta 2m+/-$  or +/+ siblings. The half lives of IgG2b in mice of the three  $\beta 2m$  genotypes were not significantly different, although degradation appeared degradation appeared more rapid in  $\beta 2m-/-$  mice.

- 11 -

Table 2. Pharmacokinetic parameters of mouse  $^{125}\text{I}$ -IgG subclass clearance.

Ig Isotype	$\beta 2\text{m}$ genotype (n)	$\text{AUC}_{\infty}^{\text{a}}$ $\times 10^7$	MRT <sup>b</sup> (hours)	Phase I half life (hours)	$t_{1/2}^{\text{c}}$ (hours)
5	IgG1	+/-, +/- (3)	10.7 (2.0) <sup>d</sup>	114 (38)	2.7 (0.4)
	IgG1	-/- (3)	3.3 (0.4)	31 (4)	3.8 (2.2)
	IgG2a	+/-, +/- (3)	2.1 (0.4)	29 (2)	2.2 (0.3)
	IgG2a	-/- (3)	1.7 (0.1)	16 (1)	2.0 (0.2)
	IgG2b	+/-, +/- (3)	1.7 (0.04)	35 (1)	1.6 (0.1)
	IgG2b	-/- (3)	1.1 (0.2)	12 (1)	2.3 (0.8)
10	IgG3	+/-, +/- (3)	8.4 (0.5)	97 (9)	2.7 (0.2)
	IgG3	-/- (2)	2.5 (0.3)	16 (2)	4.4 (1.8)
15					

<sup>a</sup> = Area under the curve<sup>b</sup> = Mean residence time<sup>c</sup> = Terminal half life<sup>d</sup> = Mean (s.e.m.)Example 2: Generation and Screening of IgG mutants.

Five amino acid residues (Ile 253, His 310, Gln 311, His 433, and Asn 434) in the conserved portion of IgG have been identified as being important in FcRn 20 binding. These are shown in Figure 2, which is a diagram showing alignment of partial amino acid sequences of immunoglobulin molecules used in Example 1 (EU numbering). Alternative amino acid residues within an isotype are shown below the most common sequence (Kabat 25 et al., 1991, Sequences of proteins of immunological interest, 5th edn., pp. 683, 692, NIH, Bethesda, MD). Predicted contacts (Burmeister et al., 1994, Nature:372:379) with FcRn are underlined. Residues required for the protection of IgG from rapid degradation 30 and for binding to FcRn (Kim et al., 1994, Eur. J. Immunol. 24:2429; Raghavan et al., 1995, Biochemistry 34:14649) are marked with an asterisk. Other amino acids can be identified using known methods.

To create a mutant IgG molecule, a cDNA encoding 35 the expressed IgG Fc-binding fragment is cloned from IgG hybridoma cells. Site-directed mutagenesis is used to

- 12 -

substitute specific amino acid residues in positions 248 through 257, 308 through 314, and 429 through 436, using a commercially-available DNA primer-based site-directed mutagenesis kit (Amersham, Arlington Heights, Illinois),  
5 according to the manufacturer's instructions.

The binding affinity of the mutant IgG antibodies to FcRn can be tested by immobilizing FcRn on a solid substrate e.g., a Sepharose™ bead, by standard methods. An anti-IgG monoclonal antibody (of other than IgG 10 isotype) is labelled with  $^{125}\text{I}$  (Dupont, Wilmington, DE) using the Iodogen™ method (Pierce Biochemical, Rockford, IL). Free iodine is removed by gel filtration on Sephadex G-200. The immobilized FcRn is contacted with 0.5  $\mu\text{g}/\text{ml}$  of the test IgG plus the  $^{125}\text{I}$ -labeled antibody 15 (e.g. for 16-18 hours at 37°C), then washed. The amount of radioactivity remaining associated with the immobilized FcRn is measured, and the binding affinity calculated using well known methods. Alternatively, binding affinity may be evaluated using an ELISA assay 20 known in the art.

Further testing in a cell-based system may also be carried out. Confluent layers of FcRn-expressing cells (e.g., cells transfected with a vector encoding FcRn) are incubated with 0.4  $\mu\text{g}/\text{ml}$   $^{125}\text{I}$ -labeled native or mutant IgG 25 to allow IgG-FcRn binding (e.g., overnight 16-18 hours at 37°C), and washed with medium (complete RPMI, 10% FCS; Gibco, Grand Island, NY). Cells are then detached by incubation with 5 mM Na<sub>2</sub>EDTA in 50 mM phosphate buffer (pH 7.5) for 5 minutes. The cells are pelleted and 30 resuspended in 2 ml 2.5 mg/ml CHAPS, 0.1 M Tris-HCl (pH 8.0), 0.3 mg/ml PMSF, 25  $\mu\text{g}/\text{ml}$  pepstatin and 0.1 mg/ml aprotinin and incubated for 30 minutes at room 35 temperature. The suspension is centrifuged at 12,000 x g for 30 minutes and the amount of radioactivity in pellets and supernatants determined as an indication of the level

- 13 -

of binding of the test IgG to FcRn. Alternatively, the test IgG can be unlabelled, and its binding detected with a labelled anti-IgG antibody as described above.

Final studies on *in vivo* clearance of the mutant IgG molecules relative to the native IgG are conducted as described in Example 1.

Example 3: Therapeutic use of IgG with increased or decreased rate of clearance.

For administration to human patients, mutant IgG molecules can be humanized by methods known in the art, e.g., monoclonal antibodies can be commercially humanized (Scotgene, Scotland; Oxford Molecular, Palo Alto, CA). Mutant IgG molecules can be purified using known methods, such as absorption onto immobilized Protein A or immunoaffinity chromatography. Following purification, the mutant IgG molecules of the invention or immunologically active fragments thereof can be administered to patients in a pharmaceutically acceptable excipient such as physiological saline. The mutant IgG molecules or other compounds of the invention, e.g., mutant IgG molecules linked to therapeutic agents such as cytotoxic moieties (e.g., radionuclides or toxic polypeptides such as ricin, *Pseudomonas* exotoxin A, and diphtheria toxin), can be administered by any standard route including intraperitoneally, intramuscularly, subcutaneously, or intravenously. It is expected that the preferred route of administration will be intravenous. These compounds can be administered systemically to the bloodstream.

As is well known in the medical arts, dosages for any one patient depends on many factors, including the patients general health, sex, size, body surface area, age, as well as the particular compound to be administered, time and route of administration, and other

- 14 -

drugs being administered concurrently. Dosages for compounds of the invention will vary, but a preferred dosage for intravenous administration is approximately 1  $\mu$ g to 500  $\mu$ g/ml/blood volume. Determination of correct 5 dosage for a given application is well within the abilities of one of ordinary skill in the art of pharmacology. The optimal dosage may be adjusted according to the condition of the patient and response of the patient to therapy.

10 Where the IgG carries a cytotoxic moiety, as for cancer chemotherapy, rapid clearance would be desired and so mutant IgGs with reduced FcRn binding are chosen. For therapeutic use of nontoxic IgG molecules, a decreased clearance rate is desired, and so mutant IgG molecules 15 with increased affinity for FcRn are selected.

Example 4: Diagnostic use of IgG with increased rate of clearance. Labelled IgG molecules of the invention can be used diagnostically. In these situations, increased clearance of IgG is desirable so that nonspecifically 20 bound labelled antibody is quickly removed from the body, thereby reducing background. Hybridoma strains producing IgG molecules specific for target tissue, e.g., cancer cells, are used, and the IgG molecules are mutated as described in Example 2. Testing for clearance is also 25 conducted as in Example 2. IgG molecules with decreased binding to FcRn, compared to wild type molecules, are chosen.

#### Other Embodiments

It is to be understood that while the invention 30 has been described in conjunction with the detailed description thereof, that the foregoing description is intended to illustrate and not limit the scope of the

- 15 -

invention, which is defined by the scope of the appended claims.

Other aspects, advantages, and modifications are within the scope of the following claims.

- 16 -

SEQUENCE LISTING

(1) GENERAL INFORMATION

(i) APPLICANT: Jacobowitz Israel, Esther  
Simister, Neil E.

5 (ii) TITLE OF THE INVENTION: MODULATION OF IgG  
BINDING TO FcRn

(iii) NUMBER OF SEQUENCES: 15

(iv) CORRESPONDENCE ADDRESS:

10 (A) ADDRESSEE: Fish & Richardson P.C.  
(B) STREET: 225 Franklin Street  
(C) CITY: Boston  
(D) STATE: MA  
(E) COUNTRY: USA  
(F) ZIP: 02110-2804

15 (v) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: Diskette  
(B) COMPUTER: IBM Compatible  
(C) OPERATING SYSTEM: DOS  
(D) SOFTWARE: FastSEQ Version 2.0

20 (vi) CURRENT APPLICATION DATA:

(A) APPLICATION NUMBER: 00786/360002  
(B) FILING DATE:  
(C) CLASSIFICATION:

25 (vii) PRIOR APPLICATION DATA:  
(A) APPLICATION NUMBER: 60/031,607  
(B) FILING DATE: November 27, 1996

(viii) ATTORNEY/AGENT INFORMATION:

30 (A) NAME: Fraser, Janis K  
(B) REGISTRATION NUMBER: 34,819  
(C) REFERENCE/DOCKET NUMBER: 00786/360002

(ix) TELECOMMUNICATION INFORMATION:

(A) TELEPHONE: 617-542-5070  
(B) TELEFAX: 617-542-8906  
(C) TELEX:

35 (2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 10 amino acids  
(B) TYPE: amino acid

- 17 -

(C) STRANDEDNESS: N/A  
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

5 Lys Asp Val Leu Thr Ile Thr Leu Thr Pro  
1 5 10

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 7 amino acids  
(B) TYPE: amino acid  
10 (C) STRANDEDNESS: N/A  
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Ile Met His Gln Asp Trp Leu  
1 5

15 (2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 8 amino acids  
(B) TYPE: amino acid  
20 (C) STRANDEDNESS: N/A  
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

His Glu Gly Leu His Asn His His  
1 5

(2) INFORMATION FOR SEQ ID NO:4:

25 (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 10 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: N/A  
(D) TOPOLOGY: linear

30 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Lys Asp Val Leu Met Ile Ser Leu Ser Pro  
1 5 10

(2) INFORMATION FOR SEQ ID NO:5:

- 18 -

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 7 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: N/A
- (D) TOPOLOGY: linear

5

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

Ile Gln His Gln Asp Trp Met  
1 5

(2) INFORMATION FOR SEQ ID NO:6:

10

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 8 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: N/A
- (D) TOPOLOGY: linear

15

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

His Glu Gly Leu His Asn His Leu  
1 5

(2) INFORMATION FOR SEQ ID NO:7:

20

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 10 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: N/A
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

25

Lys Asp Val Leu Met Ile Ser Leu Thr Pro  
1 5 10

(2) INFORMATION FOR SEQ ID NO:8:

30

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 7 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: N/A
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

Ile Gln His Gln Asp Trp Met  
35 1 5

- 19 -

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 8 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: N/A
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

His Glu Gly Leu Lys Asn Tyr Tyr  
1 5

10 (2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 10 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: N/A
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

Lys Asp Ala Leu Met Ile Ser Leu Thr Pro  
1 5 10

20 (2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 7 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: N/A
- (D) TOPOLOGY: linear

25 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

Ile Gln His Gln Asp Trp Met  
1 5

(2) INFORMATION FOR SEQ ID NO:12:

30 (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 8 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: N/A
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

- 20 -

His Glu Ala Leu His Asn His His  
1 5

(2) INFORMATION FOR SEQ ID NO:13:

5 (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 10 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: N/A  
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

10 Pro Gly Glu Leu Tyr Ile Ser Leu Asp Ala  
1 5 10

(2) INFORMATION FOR SEQ ID NO:14:

15 (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 7 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: N/A  
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

20 Val Ser Thr Gln Asp Trp Leu  
20 1 5

(2) INFORMATION FOR SEQ ID NO:15:

25 (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 8 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: N/A  
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

His Glu Ala Leu Pro Met Arg Phe  
1 5

- 21 -

What is claimed is:

1. A non-naturally occurring IgG molecule having an altered amino acid sequence compared to native IgG, and which binds to FcRn with increased affinity relative

5 to native IgG, the alteration comprising an addition or deletion of an amino acid within the FcRn-binding region of native IgG, or a substitution of an amino acid within said region.

2. A non-naturally occurring IgG molecule having 10 an altered amino acid sequence compared to native IgG,

and which binds to FcRn with decreased affinity relative to native IgG, the alteration comprising an addition or deletion of an amino acid within the FcRn-binding region of native IgG, or a substitution of an amino acid within 15 said region.

3. The IgG molecule of claim 1, in which at least one of the following amino acids conserved in native IgG is substituted with another amino acid: Ile 253, His 310, Gln 311, His 433, Asn 434.

20 4. The IgG molecule of claim 2, in which at

least one of the following amino acids conserved in native IgG is substituted with another amino acid: Ile 253, His 310, Gln 311, His 433, Asn 434.

5. A purified preparation of the IgG of claim 1.

25 6. A purified preparation of the IgG of claim 2.

- 22 -

7. A method of therapy comprising identifying an animal having a condition treatable with IgG and administering a therapeutically effective amount of the molecule of claim 1 to the animal.

5 8. A method of therapy comprising identifying an animal having a condition treatable with IgG and administering a therapeutically effective amount of the molecule of claim 2 to the animal.

10 9. The method of claim 7, wherein said condition is selected from the group consisting of idiopathic thrombocytopenic purpura (ITP), Kawasaki disease, acquired immunodeficiency syndrome (AIDS), Guillain-Barre Syndrome, and dermatomyositis.

15 10. A composition comprising the molecule of claim 2 complexed to a cytotoxic moiety.

11. The composition of claim 10, wherein the cytotoxic moiety is a toxic polypeptide or a radionuclide.

20 12. A composition comprising the molecule of claim 2, wherein the molecule comprises a cytotoxic radionuclide.

25 13. A therapeutic method comprising identifying an animal in need of treatment with an immunotoxin, and administering a therapeutically effective amount of the molecule of claim 10 to the animal.

- 23 -

14. A method of imaging a particular tissue in an animal, comprising

(a) providing the IgG molecule of claim 2, wherein the IgG molecule binds preferentially to said tissue and 5 is detectably labelled; and

(b) administering the IgG molecule to the animal in an amount effective to permit the tissue to be imaged.

15. A method for identifying an IgG molecule with an altered half-life in circulation relative to native 10 IgG, comprising

(a) contacting a molecule of FcRn with a candidate non-native IgG *in vitro*, and

(b) determining whether the candidate non-native IgG binds to FcRn with an affinity higher or lower than 15 that of native IgG, wherein an affinity higher than that of native IgG indicates that the candidate non-native IgG has a half life in circulation greater than that of native IgG, and an affinity lower than that of native IgG indicates that the candidate non-native IgG has a half 20 life in circulation shorter than that of native IgG.

16. The method of claim 15, wherein the FcRn is soluble FcRn.

17. The method of claim 15, wherein the FcRn is bound to a cell.

25 18. A method of increasing the rate of clearance of circulating IgG, comprising

(a) identifying an animal in need of an increased rate of clearance of circulating IgG, and

30 (b) administering soluble FcRn to the animal in an amount sufficient to increase the rate of clearance of circulating IgG.

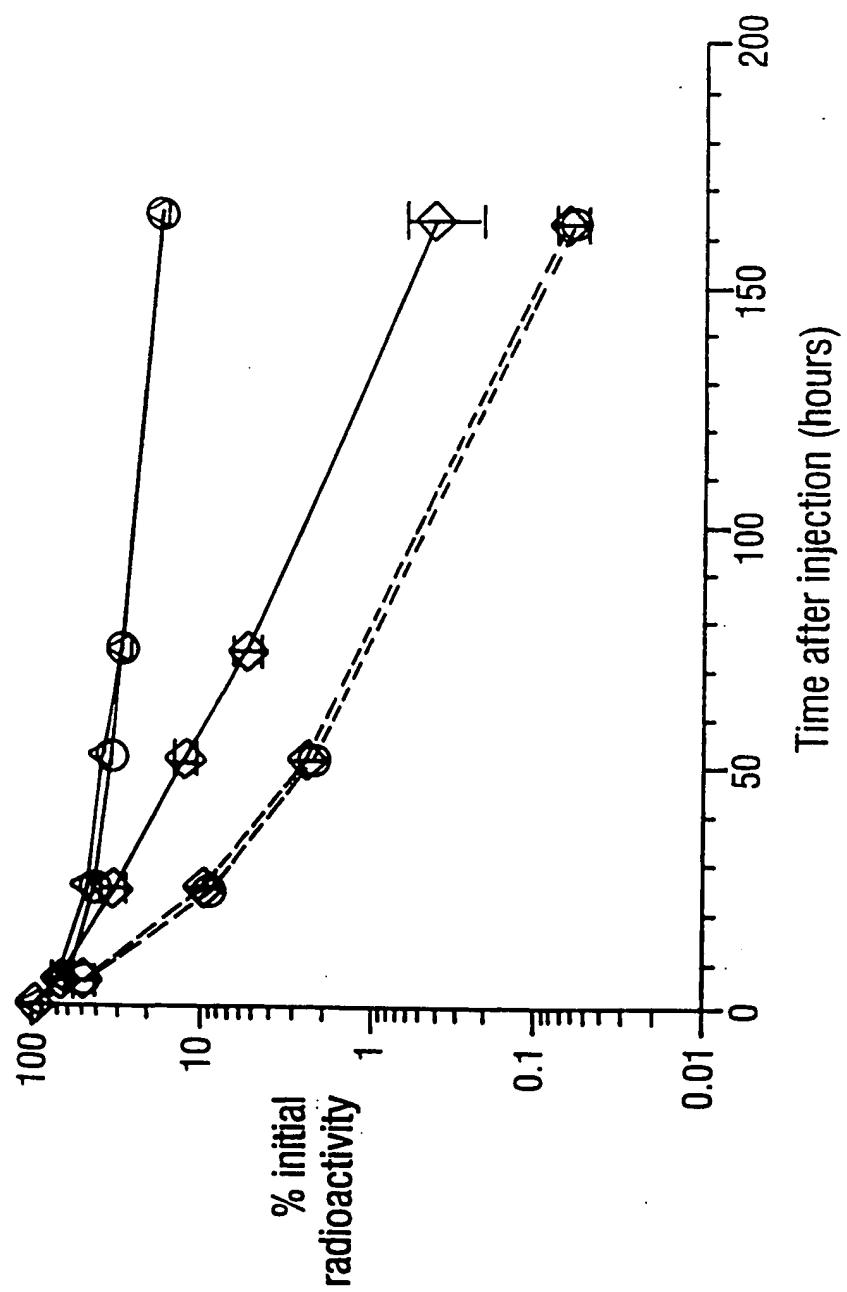
- 24 -

19. The method of claim 18, comprising administering IgG to the animal concurrently with the soluble FcRn.

20. The method of claim 18, wherein the 5 circulating IgG comprises IgG complexed with a cytotoxic moiety.

21. The method of claim 18, wherein the circulating IgG is a tumor imaging agent.

1/4



2/4

mIgG1      248      Lys Asp Val Leu Thr Ile <sup>\*</sup> Thr Leu Thr Pro      257      (SEQ ID NO: 1)  
                     Thr      Leu      Val

mIgG1      308      \*      \*      L      314      S      F      (SEQ ID NO: 2)  
                     Ile Met His Gln Asp Trp Leu

mIgG1      429      \*      \*      436      His Glu Gly Leu His Asn His His      (SEQ ID NO: 3)

mIgG2a      248      Lys Asp Val Leu Met Ile Ser Leu Ser Pro      257      (SEQ ID NO: 4)  
                     Asn

mIgG2a      308      314      Ile Gln His Gln Asp Trp Met      (SEQ ID NO: 5)

mIgG2a      429      436      His Glu Gly Leu His Asn His Leu  
                     Val      His      (SEQ ID NO: 6)

mIgG2b      248      Lys Asp Val Leu Met Ile Ser Leu Thr Pro      257      (SEQ ID NO: 7)  
                     Ser

mIgG2b      308      314      Ile Gln His Gln Asp Trp Met      (SEQ ID NO: 8)

mIgG2b      429      436      His Glu Gly Leu Lys Asn Tyr Tyr      (SEQ ID NO: 9)

mIgG3      248      Lys Asp Ala Leu Met Ile Ser Leu Thr Pro      257      (SEQ ID NO: 10)

mIgG3      308      314      Ile Gln His Gln Asp Trp Met      (SEQ ID NO: 11)

mIgG3      429      436      His Glu Ala Leu His Asn His His      (SEQ ID NO: 12)

FIG. 2A

3/4

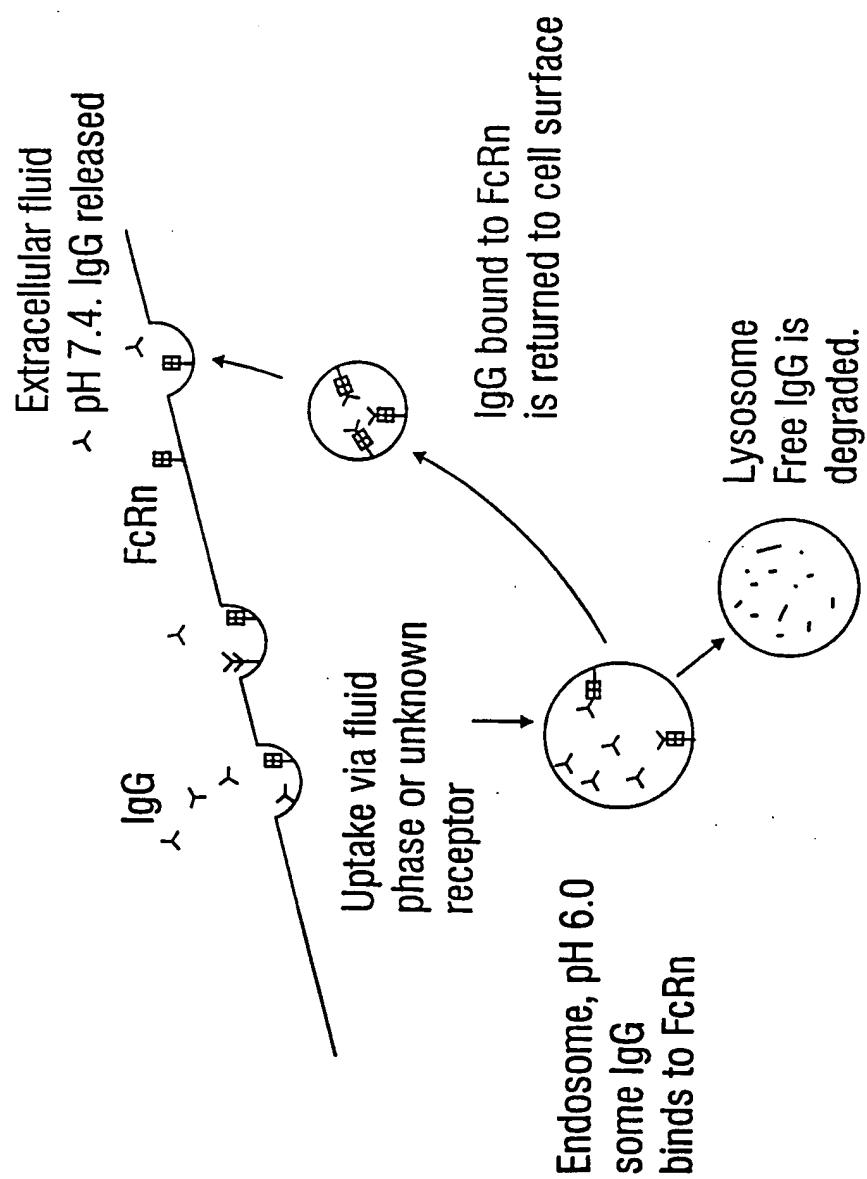
cIgY      248      Pro Gly Glu Leu Tyr Ile Ser Leu Asp Ala      257      (SEQ ID NO: 13)

cIgY      308      Val Ser Thr Gln Asp Trp      314      Leu      (SEQ ID NO: 14)

cIgY      429      His Glu Ala Leu      436      Pro Met Arg Phe      (SEQ ID NO: 15)

## FIG. 2B

4/4

**FIG. 3**

## INTERNATIONAL SEARCH REPORT

International application No.

PCT/US97/21437

## A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :A61K 39/395; C07K 16/00

US CL :424/130.1, 133.1, 143.1; 530/387.1, 387.3, 388.1, 388.22

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/130.1, 133.1, 143.1; 530/387.1, 387.3, 388.1, 388.22

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched  
Examiner's Antibody files.

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, DIALOG (file=medline), key words: antibod? FcRn, neonate

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
T	US 5,623,053 A (GASTINEL et al.) 22 April 1997, see entire document.	1-21
X	RAGHAVAN, M. et al., Analysis of the pH dependence of the neonatal Fc receptor/Immunoglobulin G interaction using antibody and receptor variants, Biochemistry 1995, Vol. 34, pages 14649-14657, see entire document.	1-2
---		-----
Y	SIMISTER, N. E. et al., An Fc receptro structurally-related to MHC Class I antigens, Nature, 12 January 1989, Vol. 337, pages 184-187, see entire document.	1-21
Y		1-21

 Further documents are listed in the continuation of Box C.

See patent family annex.

Special categories of cited documents:	*T*	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
*A* document defining the general state of the art which is not considered to be of particular relevance		
*E* earlier document published on or after the international filing date	*X*	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
*I* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	*Y*	document of particular relevance, the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
*O* document referring to an oral disclosure, use, exhibition or other means	*&*	document member of the same patent family
*P* document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search

09 MARCH 1998

Date of mailing of the international search report

16 APR 1998

Name and mailing address of the ISA/US  
Commissioner of Patents and Trademarks  
Box PCT  
Washington, D.C. 20231

Facsimile No. (703) 305-3230

Authorized officer

THOMAS CUNNINGHAM

Telephone No. (703) 308-0196

## INTERNATIONAL SEARCH REPORT

International application No.

PCT/US97/21437

## C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	STORY, C. M. et al., A major histocompatibility complex Class I-like Fc receptor cloned from human placenta: possible role in transfer of immunoglobulin G from mother to fetus, J. Exp. Med., December, 1994, Vol. 180, pages 2377-2381, see entire document.	1-21